

Irish Journal of Agricultural and Food Research **52**: 185–196, 2013

Protocols and strategies to study the migration of veterinary drug residues into milk and dairy products in licensed trials

C. Power^{1,2}, R. Sayers³, B. O'Brien³, A. Furey², M. Danaher⁴ and K. Jordan^{1†}

¹*Food Safety Department, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*

²*Team Elucidate, Department of Chemistry, Cork Institute of Technology, Bishopstown, Cork, Ireland*

³*Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*

⁴*Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland*

In the interest of animal welfare, and in order that the results from animal trials are considered valid for inclusion in the development of regulations, it is necessary that such trials are undertaken in accordance with the appropriate licensing arrangements. In January 2013, new licensing arrangements were introduced in the European Union. The aim of this paper is to outline the legislative strategy required for obtaining licences for animal trials and based on live animal trials with flukicides, establishes a blueprint for obtaining the appropriate licences and undertaking the experiments.

Keywords: Antibacterial; anthelmintics; dairy products; flukicides; licensed trials; milk; residues; veterinary drugs

Introduction

When undertaking studies involving live animals, it is important that they are undertaken in accordance with approved legislative protocols. In that way, such studies are regulated and the results can provide data to inform decision-makers. This paper, based on the protocol for obtaining licenses and undertaking trials

with veterinary drugs, attempts to set a benchmark for undertaking animal trials and to give guidance on the protocols that should be followed in research experimentation.

The research question relates to the determination of the presence and rate of withdrawal of anthelmintic and antibacterial drugs in milk, following treatment

†Corresponding author: Dr. Kieran Jordan, Tel.: +353 25 42451; Fax: +353 25 42340;
E-mail: kieran.jordan@teagasc.ie

of live animals, and their transfer to a range of dairy products during the manufacturing process. The additional questions related to (i) the pasteurisation of milk and whether the pasteurisation process results in significantly different residue levels in dairy products compared to unpasteurised milk and (ii) whether the residues are stable during storage/ripening of the manufactured products and are stable when frozen. These research questions were addressed at each stage of the process for both milk and the milk products, which included; cheese, butter and milk powder manufactured from the milk containing the analyte for each respective trial.

What are Veterinary Drugs?

Veterinary drugs are pharmacologically active substances used in the prevention, diagnosis and treatment of disease, disorder and injury in animals (Stolker and Brinkman 2005). The use of veterinary drugs in the European Union (EU) is regulated by European Council regulation (EC No. 470/2009, EU No. 37/2010; Anon. 2009; Anon 2010a). Regulation EC No. 470/2009 describes the procedure for the establishment of maximum residue limits (MRLs). The MRL is the maximum concentration of residue accepted by the EU in a food product obtained from an animal that has received a veterinary medicine or that has been exposed to a biocidal product for use in animal husbandry. The EU requires by law that foodstuffs, such as meat, milk or eggs, obtained from animals treated with veterinary medicines or exposed to biocidal products used in animal husbandry must not contain any residue that might represent a hazard to the health of the consumer. Regulation EC No. 470/2009 (Anon. 2009) describes the procedure for the establishment of MRLs.

The Annexes of regulation EU No. 37/2010 (Anon. 2010a) present the following information:

- Annex I includes substances for which final MRLs have been established;
- Annex II includes substances for which it is not considered necessary for the protection of public health to establish MRL values;
- Annex III includes substances with provisional MRLs. This includes medicines for which MRLs can be established, but clarifications from additional research studies are required before final MRLs can be set; and
- Annex IV includes substances for which no MRL can be established as residues of these substances, at whatever limit, in foodstuffs of animal origin constitute a hazard to the health of the consumer. The products in this group are prohibited for use in food producing animals in the EU.

No new medicine can be licensed or sold for use in food producing animals until its active ingredient(s) have been entered into Annexes I, II or III of Regulation EU No. 37/2010 (Anon. 2010a). In addition, Council Directive 96/23/EC (Anon. 1996) specifically regulates control and monitoring of pharmacologically active compounds (Stolker and Brinkman 2005). Residues of such compounds are divided into Group A compounds, i.e. prohibited substances in conformity with Annex IV of Regulation EU No. 37/2010 (Anon. 2010a) and Group B compounds, i.e. all registered veterinary medicines in conformity with Annexes I and III of Regulation EU No. 37/2010.

MRLs and Public Health

In order to prevent unsafe levels of veterinary drug residues from entering the

human food chain, the establishment of an MRL can lead to the setting of an appropriate withdrawal period for a particular medicine. The withdrawal period for milk is the period of time that must elapse between the last administration of a medicine and consumption or use of a foodstuff derived from the treated animal to which the medicine was administered. This period ensures that the level of residue in a foodstuff is lower than the MRL and the foodstuff is therefore deemed safe for human consumption. Setting of an MRL and withdrawal period is a necessary step for all licensed medicinal drugs administered to livestock that produce food for human consumption. These withdrawal periods must be strictly observed (Veterinary Medicines Directorate [VMD] 2008) and can vary depending on the route of administration, physicochemical properties of the drug, animal species and the type of food produced (Moreno *et al.* 2005).

Antiparasitic medicines are widely used for the protection or treatment of animals against external and internal parasitic diseases (Rahman and Samad 2010; Whelan *et al.* 2010a). The study outlined in this paper related to depletion studies of anthelmintic medicines (active against internal parasites), specifically flukicides, in dairy cows, for example triclabendazole, which has activity against mature and immature liver fluke. Triclabendazole belongs to the benzimidazole drug class and is widely used in both human and veterinary medicine for the treatment of liver fluke (*Fasciola hepatica*, class Trematoda) infections (Alvarez *et al.* 2009). Other anthelmintics examined were closantel and rafoxanide. Both are broad spectrum salicylanilide anthelmintics (Yeung *et al.* 2010) and are effective against mature and developmental stages of a number of hematophagous nematodes, trematodes

and arthropods in sheep, goats and cattle (Michels, Meuldermanns and Heykants 1987; Ghoneim *et al.* 2006). Closantel binds strongly to plasma proteins, which serves to prolong anthelmintic activity for up to 28 days (Hennessy *et al.* 1993; Yeung *et al.* 2010) and has been issued a provisional MRL until 2014 in animals producing milk for human consumption and has thus been added to Annex III of Regulation EU No. 37/2010 (Anon. 2010a).

However, rafoxanide is currently not permitted for treatment of animals where the milk is intended for human consumption due to lack of an MRL and milk withdrawal period. If sufficient data is generated on rafoxanide in the future, it also has the potential to be added to Annex III.

In addition to anthelmintics, a single broad-spectrum antibiotic, florfenicol, was investigated. Florfenicol belongs to the same amphenicol pharmacological group as chloramphenicol (Schwarz *et al.* 2004; Ruiz *et al.* 2010) and is effective as a bacteriostatic antibacterial, preventing protein synthesis in bacterial cells (Atef *et al.* 2010; Lim *et al.* 2010; Xie *et al.* 2011). However, while the mechanism of antibacterial activity is similar between florfenicol and chloramphenicol (Atef *et al.* 2010) as protein synthesis inhibitors (Sun *et al.* 2004), florfenicol has superior antibacterial activity (Ruiz *et al.* 2010) because of the presence of a fluorine atom making it resistant to deactivation by transmissible plasmids of bacteria (Ruiz *et al.* 2010). In addition, due to its lipophilicity, florfenicol demonstrates good tissue penetration (Schwarz *et al.* 2004) and is active against bovine respiratory disease (BRD) and many chloramphenicol-resistant bacterial strains (Atef *et al.* 2010). Due to its superior antibacterial activity, florfenicol is widely used in non-lactating cattle. It is not currently permitted for use in lactating

dairy cows but completion of studies on this antibiotic could lay the basis for its inclusion as a dairy animal medicine.

The Need for Data in the establishment of MRLs

There is a need for data to inform the European Medicines Agency's Committee on Veterinary Medicinal Products in the establishment of MRLs of such products in milk. Some flukicides such as closantel, nitroxylnil and triclabendazole which previously had no MRL for milk have recently been assigned provisional MRLs in milk by the European Medicines Agency's Committee on Veterinary Medicinal Products (CVMP). As these are provisional MRLs there is an obvious need for more data on the persistence of flukicide residues in milk following treatment and on the migration of these medicines into milk-derived products. These provisional MRLs, due to expire in January 2014, were set for the flukicides; nitroxylnil (Anon. 2012a), closantel (Anon. 2012b), triclabendazole (Anon. 2012c) and clorsulon (Anon. 2012d). The CVMP recommended a two-year period to allow for the completion of scientific studies on these four flukicides. There was insufficient data in order to set a provisional MRL for rafoxanide.

Animal Welfare – Licensed Trials

In Ireland, the use of animals for experimental purposes has been controlled using a statute enabled in 1876 Cruelty to Animals Act (Government of Ireland 1876; [Anon. 1876]), which has been subsequently amended by Statutory Instrument (SI) 566/2002 (Anon. 2002). This required that any person wishing to use an animal for a scientific purpose must have an authorisation (a license) issued by the

Minister for Health and Children. Prior to the 2002 amendment of the Cruelty to Animals Act by S.I. 566/2002 (Anon. 2002), the European Council of Ministers adopted Directive 86/609/EEC (Anon. 1986) on 'the protection of animals used for experimental and other scientific purposes'. It was recently decided however, that the European Directive concerning the use of animals in research should be revised and 2010/63/EU (Anon. 2010b) was applied across the EU from 1st January 2013. This Directive was transposed into Irish law by SI No. 543 of 2012 (Anon. 2012e). From January 1st 2013, the Irish Medicines Board (Anon. 2012e) became the competent authority responsible for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes in accordance with the requirements of SI No 543 of 2012 (Anon. 2012e).

Old Licensing System

Directive 86/609/EEC (Anon. 1986) sought to improve the controls on the use of laboratory animals and set minimum standards for housing and for the training of personnel in handling animals for scientific purposes. In addition, the Directive involved the supervision of animal experiments with the objective of reducing the numbers of animals used for experimentation by: (i) requiring that an animal experiment should not be performed if an alternative method existed and (ii) by encouraging the development and validation of alternative methods to replace animal methods. The Directive envisaged that most experiments would be carried out while the animal was under anesthetic and that it would not be allowed to recover from that state, i.e. the animal would be euthanised. However, it was recognised that there would be instances where the

immediate death of the animal would frustrate the aims of the experiment. In such cases, there was provision within the Act for the granting of certificates (or exemptions), such as:

- Certificate A – permitted the worker to dispense with anaesthesia where the minimum amount of pain was envisaged. (This was the exemption sought and granted for use in the trials in this current study);
- Certificate B – permitted an animal to recover from an anaesthetic where the post-procedural care of the animal was required to be given in detail in order to acquire the certificate;
- Certificate C – permitted the use of animals in the teaching of students;
- Certificate D – necessary if the animal could potentially experience severe pain that was likely to be prolonged;
- Certificates E, EE & F – necessary if dogs, cats or equines were to be used as experimental animals; and
- Certificate G – necessary whenever the setting-free of the animal was necessary for the legitimate purposes of the proposed experiment.

In addition to the license and certificate required, the Minister of Health and Children also required two prominent members of society, for example, a professor of surgery/medicine or a medical practitioner to add their approval to the licence. These signatories were required to certify that the aim of the project was worthwhile and could not be produced by anaesthetics without necessarily undermining the objective of the experiment.

As a standard condition, it was required that the experiments were performed at a registered place approved by the Minister of Health and Children. Registered places were visited by inspectors from time to time for the purpose of ensuring

compliance with the Cruelty to Animals Act 1876 (Anon. 1876). Licensees were required to keep a written record of their experiment(s), sending a “return” of the number and nature of the experiments(s) performed during the year to the Department of Agriculture, Food and the Marine (DAFM) BioResources Services (Trinity College Dublin 2012). The licence number obtained from the Department of Health and Children in 2011 to enable completion of trials for this study was Ref: B100/4375 Expiry Date 19th December 2015. All trials in the current study were completed prior to the change in legislation. Therefore, the requirements under the Department of Health and Children and DAFM applied.

Animal Remedies License

In addition to the experimentation licensing requirement, research involving the use of unlicensed medicines requires a license from DAFM. The application to DAFM includes details of the veterinary medicine proposed for administration, information on the type of animal that is to be used for each trial and identification of the people involved in ensuring the welfare of trial animals, including the experimental license authorisation number for each person. It is imperative that this application indicates that the trial will be conducted in accordance with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products guidelines on good clinical practice (VICH GL9 on GCP) of June 2000 (as implemented in July 2001). Compliance with these guidelines provides assurance regarding the integrity of trial data and that animal welfare is guaranteed throughout the entirety of the trial. Compliance with manufacturers’ guidelines regarding

appropriate dosage procedures for the veterinary drug to be administered must be included in each license application. Finally, the procedure to be followed in cases where the trial animal develops an adverse reaction to the administered drug must be specified.

In the trials conducted in this current study, milk samples were taken from the trial animals following drug administration until residues had depleted to undetectable levels. The license numbers obtained from DAFM for each trial conducted in this study were as follows:

- DAFM License No. RL/10/03 for lactating trial for triclabendazole, closantel and rafoxanide (valid from 28/1/11 to 27/1/12);
- DAFM License No. RL/11/03 for lactating trial for florfenicol (valid from 8/8/11 to 7/8/12); and
- DAFM License No. RL/10/03A for dry period closantel trial (valid from 1/11/11 to 30/6/12).

New Licensing System

Under the new Directive 2010/63/EU (Anon. 2010b), each establishment which breeds, supplies or uses animals intended for scientific purposes must have an internal animal welfare body. This is in accordance with Article 26 of Directive 2010/63/EU and Regulation 50 of S.I. No. 543 of 2012 (Anon. 2012e), where the individuals responsible for the welfare and care of animals within each establishment are required to be a member of the animal welfare body.

The animal welfare body is required to consist of at least one person responsible for the welfare and care of animals and in the case of a user establishment, at least one member of the scientific workforce. Therefore, in practice, there must be at least two members from each

establishment on the animal welfare body. The designated veterinarian or suitably qualified expert may or may not be a member also, but in any case must provide input to the animal welfare body. The duties of the animal welfare body are set out in Article 27 of Directive 2010/63/EU (Anon. 2010b). Information on each member appointed to the animal welfare body is expected to be provided with a clear outline of their role in that body. In some establishments, the animal welfare body may be linked to the ethics committee of the establishment. If this is the case, the relationship between the two bodies is required to be described in the site master file in accordance with Regulation 36(2) of S.I. No. 543 of 2012 (Anon. 2012e) which contains information about the main activities carried out at the designated site, the quality management system in operation at the site and the lines of control and responsibilities exercised by the personnel at the site.

Directive 2010/63/EU (Anon. 2010b) lays down specific requirements for personnel involved in various procedures at the establishment through Articles 20(2), 24 and 25. Details on each of the responsible personnel in the establishment must therefore be provided, specifically:

- Compliance officer: the person responsible for compliance of the establishment with the provisions of Article 20(2) of Directive 2010/63/EU (Anon. 2010b) and Regulation 44 of S.I. No. 543 of 2012 (Anon. 2012e);
- Animal care and welfare officer: the person responsible for overseeing the welfare and care of the animals at the establishment (Article 24 [1a] of Directive 2010/63/EU and Regulation 45 of S.I. No. 543 of 2012);
- Training officer: the person responsible for ensuring that the staff are adequately educated, competent and

continuously trained in animal care and handling and that they are supervised until they have demonstrated the requisite competence (Article 24[1c] of Directive 2010/63/EU and Regulation 46 of S.I. No. 543 of 2012); and

- The designated veterinarian or suitably qualified expert in accordance with Article 25 of Directive 2010/63/EU and Regulation 48 of S.I. No. 543 of 2012.

The documentation on the monitoring of the health and welfare of animals at the establishment is required which includes the following: (i) the maintenance of animal records including records of animal mortality and (ii) ensuring the compliance with any standard operating procedures for the conducting of procedures and / or euthanasia in animals, including animal statistics on the use of animals in procedures and on the actual severity of the procedures. This documentation must be generated and returned to the competent authority on an annual basis (in accordance with Article 54). This information is mandatory. Once an establishment authorisation is granted the establishment is licensed for a maximum period of 3 years, as opposed to 5 years under the Department of Health and Children and is subject to renewal thereafter.

Each individual involved in performing experimental procedures on animals must be individually authorised by the Irish Medicines Board (IMB) before conducting any procedures. Individual authorisation is obtained by submission of the relevant form, a CV and training record. Overall project authorisation can be applied for using the relevant forms, which involves submission of a technical and non-technical version of the project proposal. Approval of each project by the research institutions' ethics committee must also be submitted with the application, unless an explanation can be provided as to why it has not been included. Finally, a request for experiment classification can be submitted in order to determine if a full experimental proposal application is required. Again, the relevant form is available from the IMB. A summary of current licensing procedures is included in Figure 1.

Experimental Design

Lactating cow trials

For triclofenazole, rafoxanide, closantel and florfenicol, four separate *in vivo* trials, where the drugs were administered individually, were conducted in the lactating period to ascertain the withdrawal period

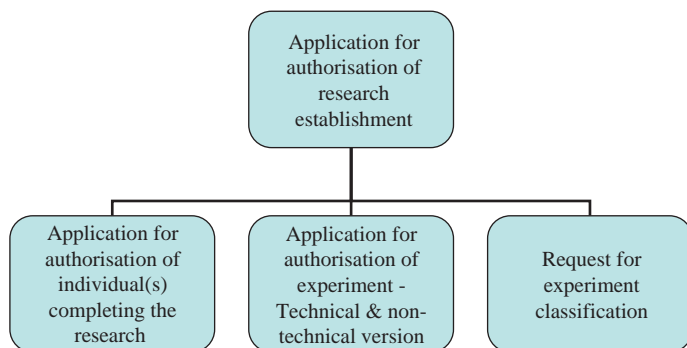


Figure 1. Summary of the new IMB experimental licensing procedures.

for each analyte. A total of six lactating dairy cows (Friesian, $n = 3$; Montbeliard, $n = 2$; Norwegian Red, $n = 1$) were assigned to the experiment. For each of the four trials, the six cows were divided into two groups of three cows (balanced by breed as much as possible) and the total milk from each group was collected and pooled on days 2 and 23, which should be representative of high and low residue concentrations, respectively. Each milk pool was further sub-divided into two; one portion was pasteurised ($72\text{ }^{\circ}\text{C} \times 15\text{ s}$) while the second remained unpasteurised, resulting in eight portions of milk for each of the four lactating cow trials. For further detail on the experimental procedure, see Power *et al.* (2013a).

Milk studies Following treatment, milk samples were taken twice daily (morning and evening) up to day 23 post-administration for the triclabendazole trial. For the closantel trial, milk samples were taken twice daily (morning and evening) up to day 59 (when concentrations were below the MRL) and subsequently samples were collected weekly until day 199 post-treatment. The rafoxanide trial required milk samples to be taken twice daily up to day 68 post-administration and for the florfenicol trial, milk samples were taken twice daily up to day 28 post-administration until no respective analyte was detected in the milk. Samples were labeled on collection and stored at $-20\text{ }^{\circ}\text{C}$ and analysed within one week of collection for each of the four trials. Since it was necessary to use the same six cows for all trials and as low residual concentrations of closantel remained for 199 days, it was necessary to begin the rafoxanide trial at day 63 when the closantel concentration was below the provisional MRL of $45\text{ }\mu\text{g/kg}$ for all six cows. This did not interfere with the detection of either compound.

Product studies From each portion of milk, a semi-soft laboratory scale cheese was manufactured and the remainder of the portion was separated into skim-milk and cream. After separation of the curd and whey during cheesemaking, both were analysed in addition to the final cheese. Butter and buttermilk were manufactured from the cream and skim-milk powder was manufactured from the skim-milk (Figure 2).

Stability studies For all trials, samples were studied by analysing fresh product on the day of manufacture, freezing it at $-20\text{ }^{\circ}\text{C}$ and analysing the samples again after 6 and 12 months. Furthermore, after each weekly sample of cheese or butter had been analysed, the remaining portion of each sample was frozen at $-20\text{ }^{\circ}\text{C}$ for further residue analysis at 6 and 12 months. Skim milk powder was stored in 50 mL centrifuge tubes in the dark at ambient temperature ($18\text{--}22\text{ }^{\circ}\text{C}$). Samples of powder were taken at day 0 and at 6 and 12 months and analysed for the presence of residues.

Dry-cow trials

For the dry period closantel trial, closantel was administered to the same six cows prior to drying off, at the end of lactation. Milk samples were taken from each individual cow following calving for up to 189 days post-administration, when residue could be no longer detected in the milk sampled from any of the six trialed animals.

Replicates

Since individual cows will metabolise veterinary drugs at a different rate, it is necessary to monitor drug depletion in the individual cows. Therefore, the milk from each of the six cows was analysed independently.

Product manufacture

Trials with triclabendazole, closantel, rafoxanide and florfenicol analytes (at

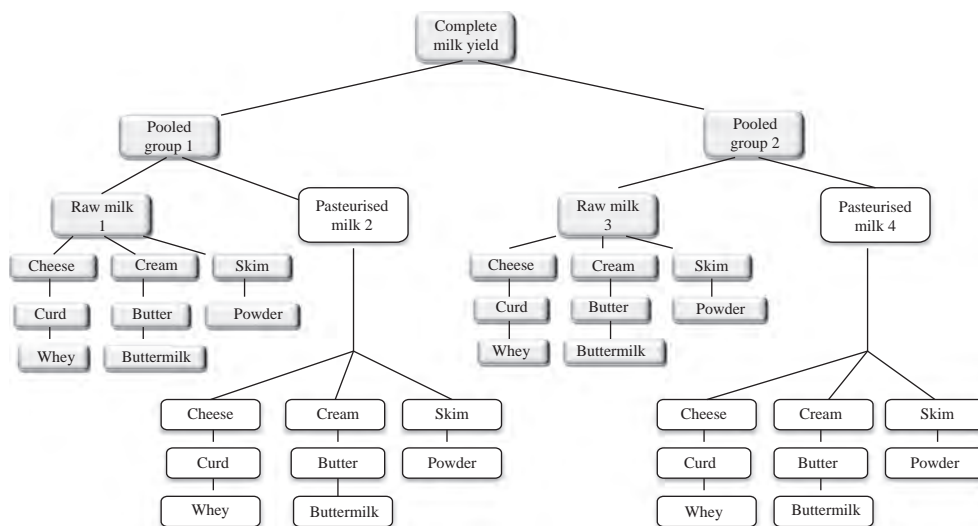


Figure 2. Flowchart of the process of product manufacture from milk for each analyte being trialed. This flow chart was followed for each of the 4 residues at high and low concentrations. Reprinted with permission from Power *et al.* (2013b). Copyright (2013) American Chemical Society.

high and low residue concentrations) were undertaken in independent duplicate, with products being manufactured from two separate pools of milk from grouped animals (Figure 2). In addition, product was made from both pasteurised and unpasteurised milk, resulting in four independent analyses (two with pasteurised milk and two with unpasteurised milk).

Validation and analyses

Residue analysis of the samples was undertaken by Ultra High Performance Liquid Chromatography – Tandem Mass Spectrometry UHPLC-MS/MS (Whelan *et al.* 2010b). The validation of the analyses in milk was carried out by fortifying negative bovine, caprine and ovine milk samples at 0.5, 1.0 and 1.5 times the provisional MRL ($n = 7$ at each level) for measurement of triclabendazole, rafoxanide, closantel and florfenicol.

The dairy product validations were carried out by fortifying negative controls

(butter manufactured from bovine milk, cheeses manufactured from bovine, caprine and ovine milk and skimmed milk powder and infant formula manufactured from bovine milk) at differing concentrations ($n = 7$ at each level) for each analyte. The results of the experiments undertaken have been published elsewhere (Power *et al.* 2012; 2013a,b,c,d).

Study Animals Post-Experimentation

As per the licensing agreement, milk from the six animals was added to the slurry tank and spread as slurry. The six animals were slaughtered on 13th December 2012, in excess of one year after the final administration of a veterinary medicine.

Summary

This protocol sets out the licensing and documentation requirements for live animal trials in terms of animal welfare

(housing and management), personnel conducting the work and the use of trial substances. A protocol such as that described here is important in that it is a good guideline to follow when undertaking licensed trials involving Department regulators.

The project was undertaken in Teagasc and represented a collaborative work involving the Teagasc Animal and Grassland Research and Innovation Centre in Fermoy, Co. Cork, (AGRIC), where animals were treated, the Teagasc Food Research Centre, Fermoy, Co. Cork (TFRCM), where product was manufactured, and the Teagasc Food Research Centre, Ashtown, Dublin (TFRCA), where analyses were undertaken, with supervision from Cork Institute of Technology.

All animal trials for each veterinary drug under investigation were fully licensed by both the Department of Health and Children and DAFM according to pre-2013 experimental license guidelines. A new licensing system for experimentation using animals came into effect on January 1st 2013.

References

- Alvarez, L., Moreno, G., Moreno, L., Ceballos, L., Shaw, L., Fairweather, I. and Lanusse C. 2009. Comparative assessment of albendazole and triclabendazole ovicidal activity on *Fasciola hepatica* eggs. *Veterinary Parasitology* **164**: 211–216.
- Anonymous. 1876. An Act to amend the Law relating to Cruelty to Animals (15th August 1876). Available online: <http://web.archive.org/web/20061214034848/http://homepage.tinet.ie/~pnowlan/Chapter-77.htm>. [accessed 3 December 2013].
- Anonymous. 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* **L358**: 1–28.
- Anonymous. 1996. Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. *Official Journal of the European Communities* **L125**: 10–32.
- Anonymous. 2002. Statutory Instruments (S.I) No. 566/2002 – European Communities (Amendment of Cruelty To Animals Act 1876) Regulations 2002. Available online: <http://www.irishstatutebook.ie/2002/en/si/0566.html>. [accessed 3 December 2013].
- Anonymous. 2009. Regulation EC. No. 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation EC. No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation EC. No. 726/2004 of the European Parliament and of the Council. *Official Journal of the European Communities* **L152**: 11–22.
- Anonymous. 2010a. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Communities* **L51**: 1–72.
- Anonymous. 2010b. Council Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Communities* **L276**: 33–79.
- Anonymous. 2012a. Commission Implementing Regulation EU No. 466/2012 of 1 June 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance clorsulon. *Official Journal of the European Communities* **L143**: 2–4.
- Anonymous. 2012b. Commission Implementing Regulation EU No. 221/2012 of 14 March 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance closantel. *Official Journal of the European Communities* **L75**: 7–9.
- Anonymous. 2012c. Commission Implementing Regulation EU No. 201/2012 of 8 March 2012 Amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as

- regards the substance nitroxylin. *Official Journal of the European Communities* **L71**: 37–39.
- Anonymous. 2012d. Commission Implementing Regulation EU No. 222/2012 of 14 March 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance triclabendazole. *Official Journal of the European Communities* **L75**: 10–11.
- Anonymous. 2012e. Statutory Instruments (S.I.) No. 543/2012 – European Union (Protection of Animals used for Scientific Purposes) Regulations 2012. Available online: <http://www.irishstatutebook.ie/2012/en/si/0543.html> [accessed 25 November 2013].
- Atef, M., El-Gendi, A.Y.I., Amer, A.M. and Abd El-Aty, A.M. 2010. Effect of three anthelmintics on disposition kinetics of florfenicol in goats. *Food and Chemical Toxicology* **48**: 3340–3344.
- Ghoneim, M.M., El-Ries, M., Hassanein, A.M. and Abd-Elaziz, A.M. 2006. Voltammetric assay of the anthelmintic veterinary drug nitroxylin in bulk form and formulation at a mercury electrode. *Journal of Pharmaceutical and Biomedical Analysis* **41**: 1268–1273.
- Hennessy, D.R., Sangster, N.C., Steel, J.W. and Collins, G.H. 1993. Comparative pharmacokinetic disposition of closantel in sheep and goats. *Journal of Veterinary Pharmacology and Therapy* **16**: 254–260.
- Lim, J.H., Kim, M.S., Hwang, Y.H., Song, I.N., Park, B.K. and Yun, H.I. 2010. Plasma and tissue depletion of florfenicol in olive flounder (*Paralichthys olivaceus*) after oral administration. *Aquaculture* **30**: 71–74.
- Michels, M., Meuldermanns, W. and Heykants, J. 1987. The metabolism and fate of closantel (Flukiver) in sheep and cattle. *Drug Metabolism Reviews* **18**: 235–251.
- Moreno, L., Imperiale, F., Mottier, L., Alvarez, L. and Lanasse, C. 2005. Comparison of milk residue profiles after oral and subcutaneous administration of benzimidazole anthelmintics to dairy cows. *Analytica Chimica Acta* **536**: 91–99.
- Power, C., Sayers, R., O'Brien, B., Bloemhoff, Y., Danaher, M., Furey, A. and Jordan, K. 2012. Partitioning of nitroxylin, oxclosanide and levamisole residues from milk to cream, skim milk and skim milk powder. *International Journal of Dairy Technology* **65**: 503–506.
- Power, C., Whelan, M., Danaher, M., Bloemhoff, Y., Sayers, R., O'Brien, B., Furey, A. and Jordan, K. 2013a. Investigation of the persistence of triclabendazole residues in bovine milk following lactating-cow and dry-cow treatments. *Food Additives and Contaminants* **30**: 1080–1086.
- Power, C., Sayers, R., O'Brien, B., Clancy, C., Furey, A., Jordan, K. and Danaher, M. 2013b. Investigation of the persistence of closantel residues in bovine milk following lactating-cow and dry-cow treatments and its migration into dairy products. *Journal of Agricultural and Food Chemistry* **61**: 8703–8710.
- Power, C., Danaher, M., Sayers, R., O'Brien, B., Clancy, C., Furey, A. and Jordan, K. 2013c. Investigation of the migration of triclabendazole residues to milk products manufactured from bovine milk, and stability therein, following lactating cow treatment. *Journal of Dairy Science* **96**: 6223–6232.
- Power, C., Danaher, M., Sayers, R., O'Brien, B., Whelan, M., Furey, A. and Jordan, K. 2013d. Investigation of the persistence of rafoxanide residues in bovine milk and fate during processing. *Food Additives and Contaminants* **30**: 1087–1095.
- Rahman, M.M. and Samad, M.A. 2010. Prevalence of subclinical gastro-intestinal parasitosis and their effects on milk production with therapeutic management in red chittagong cattle. *Bangladesh Society for Veterinary Medicine* **8**: 11–16.
- Ruiz, J.B., Zapata, M.N., López, C.C. and Gutiérrez, H. 2010. Florfenicol concentrations in milk of lactating cows posttreated by intramuscular or intramammary routes. *Revista MVZ Cordoba* **15**: 2041–2050.
- Schwarz, S., Kehrenberg, C., Doublet, B. and Cloeckaert A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews* **28**: 519–542.
- Stolker, A.A.M. and Brinkman, U.A. 2005. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals - a review. *Journal of Chromatography A* **1067**: 15–53.
- Sun, Y., Scruggs, D.W., Peng, Y., Johnson, J.R. and Shukl, A.J. 2004. Issues and challenges in developing long-acting veterinary antibiotic formulations. *Advanced Drug Delivery Reviews* **56**: 1481–1496.
- Trinity College Dublin. 2012. “BioResources Services” Available online: <http://www.tcd.ie/BioResources/licence-ethical/introduction/certificates.php> [accessed 25 November 2013].
- Veterinary Medicines Directorate. 2008. “Code of Practice on the Responsible use of Animal Medicines on the Farm”. Available online: <http://adlib.eversysite.co.uk/resources/000/020/015/RUCOP.pdf> [accessed 25 November 2013].

- Whelan, M., Chirollo, C., Furey, A., Cortesi, M.L., Anastasio, A. and Danaher, M. 2010a. Investigation of the persistence of levamisole and oxclozanide in milk and fate in cheese. *Journal of Agricultural and Food Chemistry* **58**: 12204–12209.
- Whelan, M., Kinsella B., Furey A., Moloney M., Cantwell H., Lehotay, S. and Danaher, M. 2010b. Determination of anthelmintic drug residues in milk using ultra high performance liquid chromatography-tandem mass spectrometry with rapid polarity switching. *Journal of Chromatography A* **1217**: 4612–4622.
- Xie, K., Jia, L., Yao, Y., Xu, D., Chen, S., Xie, X., Pei, Y., Bao, W., Dai, G., Wang, J. and Liu, Z. 2011. Simultaneous determination of thiamphenicol, florfenicol and florfenicol amine in eggs by reversed-phase high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B* **879**: 2351–2354.
- Yeung, H.S., Ching, W.H., Lai, S.S.L., Lee, W.O. and Wong, Y.T. 2010. Quantitative analysis of closantel and rafoxanide in bovine and ovine muscles by high-performance liquid chromatography with fluorescence detection. *Journal of AOAC International* **93**: 1672–1677.

Received 26 September 2013